CHROMOSOMAL ANALYSIS OF COSTUS SPECIOSUS (KOEN.) J. E. SMITH, A THREATENED MEDICINAL PLANT OF MELGHAT TIGER RESERVE

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ABSTRACT

Costus speciosus is commonly called as ranadrak, belonging to family Zingiberaceae. It is a threatened medicinal plant of Melghat Tiger Reserve, Maharashtra. The main alkaloid content is diosgenin and it is medicinally used against different ailments like headache, fever, cough, cuts and wounds, scabies and stomach disorder. A standard protocol was established for squashing of *in vivo* root tips. Active mitotic frequency was recorded as 2.13%. Two types of mitotic abnormalities were recorded in low numbers i.e., sticky metaphases and sticky anaphases.

Keywords: *Costus speciosus*, threatened, chromosome, mitotic frequency, abnormalities.

Introduction

Melghat Tiger Reserve (MTR) is situated in the mountainous region, the Gavilgarh hills of Satpuras from Dharni and Chikhaldara tahsils of Amravati District of Maharashtra state. The forest is of dry deciduous type and vegetation changes occur at close intervals. Tiger reserve covers the total area of 1676.93 sq kms.

C. speciosus is commonly called as Keu or ranadrak, belonging to family Zingiberaceae. Nearly 100 species of the genus *Costus* are found in the tropics of both hemispheres (Cooke, 1958). It is found throughout India from the Central and Eastern Himalayas, ascending to 4000 ft. to Ceylon and Malacca, Malay islands, China and Western Ghats (Hooker, 1894; Bombay State Gazetteer, 1953). It is indigenous to the Indo-Malayan region. It grows wild in wet places in Assam plains, foot hills of Kangra (H.P.), Khasi and Jaintia hills and coastal regions of Goa and Kerala (Sharma, 2004).

Rhizomes of *C. speciosus* Sm. have been found to contain up to 2.6% of alkaloid diosgenin (Sarin et. al. 1974; Kaphai et. al. 1977). The highest diosgenin yield (95.8 Kg/ha) was obtained with Mohanlalganj material (Lucknow) of *C. speciosus* (Sharma et. al. 1980). From the seeds of *C. speciosus*, palmitic and oleic acids, diosgenin and two unidentified triterpenoids have been isolated by Dixit and Srivastava (1987). CSS, a mixture of saponin seems to possess antifertility activity which is probably due to its abortifacient properly (Tewari et al. 1973). The tribals "Kanikkars" use this plant internally and externally for common ailments like headache, fever, cough, cuts and wounds, scabies and stomach disorder (Janaki Ammal and Prasad, 1984). Rhizomes cooked and eaten; accredited with purgative and tonic properties and roots used as a tonic and anthelmintic (Ambasta, 1994).

The world 'threatened' has been used for species which comes under one of the three categories, i.e., endangered, rare and depleted. In India, *C. speciosus* has been heavily depleted as a result of natural causes or human activity (Maheshwari, 1977). It is threatened medicinal plant recorded from Madhaulia forest of Gorakhpur and found frequently in Sal areas (Ansari, 1993). It is frequently noticed as forest undergrowth in Tadoba National Park (Malhotra and Moorthy, 1992). *C. speciosus* is among the 46 species included in the list of species banned from export by the Ministry of Commerce [Vide notification 47 (PN) / 92-97 dated 30 March 1994]. *C. speciosus* is found occasional in Semadoh, Harisal and Tarubanda ranges of Melghat Tiger Reserve in deep valleys and moist shady areas along stream and river banks (Dhore and Joshi, 1988). Presently number of this species is fastly depleted in all parts of MTR due to indiscriminate uprooting of the plants for

rhizomes which are extensively used as drug in pharmaceutical industries.

The diploid chromosome number 2n = 18 of *C. speciosus* was found in the eastern parts of India while triploids (2n = 27) in Andaman Islands. The tetraploids (2n = 36) seem to have a very wide distribution and have been collected from the eastern, northern and southern parts of India (Subrahmanyam, 1978; Subrahmanyam and Khoshoo, 1986). The main objective of the present study is to develop a protocol for mitotic squashing methodology for normal stages and chromosomal irregularities of *C. speciosus*.

Materials and Methods

Survey and collection

Survey of different forest areas of Melghat Tiger Reserve (MTR) was carried out for the collection of plant material. The plant specimens were collected both from core as well as buffer areas. The specimens of *C. speciosus* were found in the patches of both the sides of river Kuwapati near Mangia, Gadga river near Koha; along the roadside of Semadoh, Kund and Mangia, near Chikhaldara and Bhimkund valley. The number was differing from 2 to 40 in the patches at every place. The highest number of plants (40) at one spot is found near Koha, some of them were small others bigger up to 5 feet in height bear beautiful attractive flowers. They also found in the areas of thick forest of Kolkaj and Semadoh. The conditions required for the fast growth of healthy plants are high moisture, low temperature and high rainfall. The cloudy environment is very suitable for its good growth. In the humus containing wet black soil, the number of plants was found in increasing order than the muroom and rocky soil.

The tuberous rhizomes (Fig.1) found nearly one foot deep in the soil; yellowish brown in colour and 2-3 buds present on each of one. The rhizomes remain dormant for the complete year however, with coming of rainy season, start germinating. The germination period begins in the month of June and extends up to August. Flowering begins after the completion of vegetative growth, of plant. The flowers borne at the shoot tip; number many, colouration changes from white to pink having bracts of dark reddish pink (Fig.2). When flowers were weather, the colour change to brownish and then dry. The flowering period starts from August and ends up to the first week of October. After completion of reproductive phage, the shoots and leaves turned to yellow and after some day's dries but plant perenets by underground tuberous rhizome throughout the unfavorable season.

Rhizomes of *C. speciosus* were collected along with moist soil in polythene bags and maintained them in earthen pots at Garden of Botany Department. Underground tuberous material was germinated within 3-4 days and the vegetative bud emerged out and grew to a plantlet during the course of time, which differed with individual material. At the collection place, flowering part of the shoot was cut, taken in between the folds of Newspaper and pressed under wooden presser. Three herbarium specimens was prepared and preserved in the Department of Botany. The plant material was confirmed as *Costus speciosus* (Koen.) J. E. Smith, from Botanical Survey of India, Western Circle, Pune.

Squashing methodology

Standard protocol of squashing for *in vivo* study of root tip mitosis given by Sharma and Sharma (1990) in their book entitled, 'Chromosome technique: theory and practice' was followed. In present study of *in vivo* mitosis certain changes are made in protocol as per the requirements of the plant. Rhizomes were germinated by placing them on moist soil so that within a week young roots were seen to emerge. Root tips were cut in the morning hours and transferred into fixative. The peak mitotic activity of cell division was observed at 9– 10 am in morning hours. Then the root tips were fixed in Carnoy's I for 24 hrs and on thorough washing with distilled water; they were preserved in 70% alcohol.

Root tips were hydrolyzed best required higher concentration of HCl (2N) at 60°C for 10 min's. After hydrolysis, place the root tip on slide and cut the terminal meristematic region with a fine blade. Then roots tips were stain with 1% acetoorcein for 5 min at room temperature and put a cover-slip over material. The cover-slip was pressed once with thumb after placing it in between the folds of blotting paper for uniform spreading of cells. Temporary sealing was done to the temporarily prepared slides with mixture of paraffin wax. Different stages of mitosis and irregularities were observed under microscope (10X40X) and count metaphases, anaphases, and telophases and then calculate frequency. And photomicrograph was taken on trinocular microscope (Carl Zeiss).

Calculations

The mitotic frequency of different phases were calculated by using following formulas

$$\text{Active mitotic frequency (\%)} = \frac{\text{Equatorial Met + Anaphase}}{\text{Total number of cells scored}} \times 100$$

$$\text{Mitotic abnormality (\%)} = \frac{\text{Total number of abnormalities}}{\text{Total number of cells scored}} \times 100$$

$$\text{Mitotic frequency (\%) of Phases (e.g. Metaphase)} = \frac{\text{Number of metaphases}}{\text{Total number of cells scored}} \times 100$$

Results

The root tip fixation, hydrolysis and staining procedure was giving excellent results by imparting bright pink – purple colour to chromosomes for clear observation of different normal and abnormal mitotic stages. The mitotic frequency of normal stages and mitotic abnormalities in *in* viv*o* plantlets were calculated in five root tips from different plants (Table). Frequencies of polar metaphases (Fig.3) and equatorial metaphases, anaphases and telophases were ranging from 0.94 – 1.48%; 0.77 - 1.96%; 0.35 – 1.47% and 1.06 – 2.32% respectively. Active mitotic frequency was recorded as 2.13%. After individual root mitotic analysis was carried out, maximum frequency of telophases (2.32%) recorded in root 2; equatorial metaphases (1.96%) in 1st; polar metaphases (1.48%) in 3rd and anaphases (1.47%) in 5th root. Hence from the results, it appeared that all the five roots showing normal division except negligible number of sticky metaphases (Fig.4) and sticky anaphases (Fig.5) scored in 5th root.

Root	Frequency of mitosis number (%)					Mitotic abnormalities number (%)
	Cells scored	Polar metaphase	Equatorial metaphase	Anaphase	Telophase	Sticky metaphase
1st	1377	13 (0.94)	27 (1.96)	11 (0.79)	24 (1.74)	-
2nd	902	9 (0.99)	10 (1.10)	11 (1.21)	21 (2.32)	-
3rd	1410	21 (1.48)	15 (1.06)	5 (0.35)	17 (1.20)	-
4th	1415	20 (1.41)	11 (0.77)	17 (1.20)	15 (1.06)	-
5th	1087	14 (1.28)	9 (0.82)	16 (1.47)	15 (1.37)	1 (0.09)

Fig.1 Germinating tuber

Fig.2 Flowering twig with unopened buds

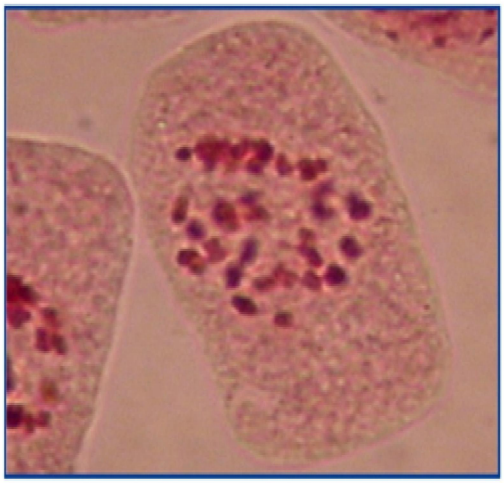

Fig.3 Polar metaphase

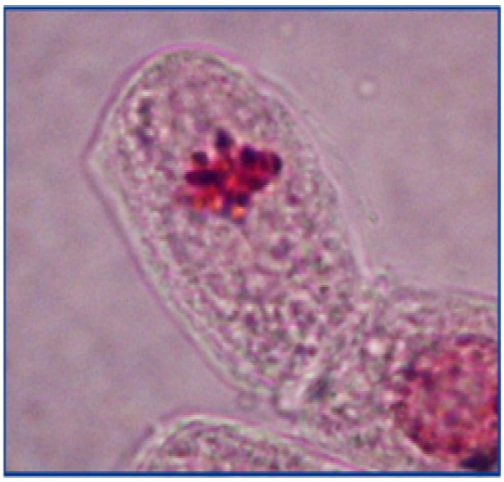

Fig.4 Sticky metaphase

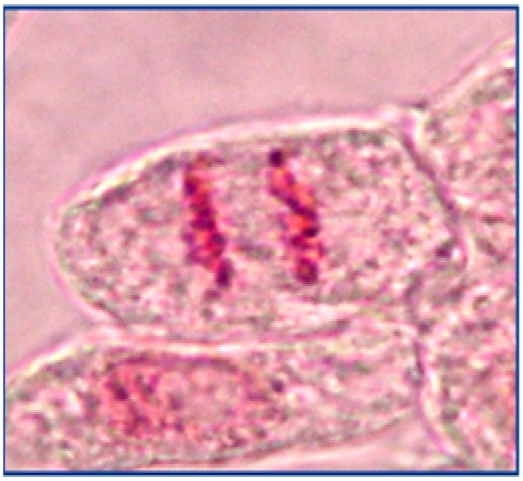

Fig.5 Sticky anaphase

Discussion

Present study showed that root tips of *C. speciosus* were well hydrolyzed with 2N HCl at 60˚C for 10 min and staining with 1% acetoorcein for 5 min. Subrahmanyam and Khoshoo (1986) hydrolyzed root tips with 1N HCl at 60˚C for 12 min however, they used Feulgen stain. Tyagi and Gupta (1987) have macerated root tips of *C. speciosus* in 1N HCl at 58 – 60˚C for 10 – 15 min and staining with 2% aceto carmine + N HCl (9:1) mixture. Chattopadhyay and Sharma (1983) hydrolyzed root tips by using 5N HCl at 18 - 20˚C for 15 min, stained with acetoorcein -(N)HCl mixture (9:1) in *C. speciosus*. It appears that the root tips of *C. speciosus* are not that much soft to macerate as evident by earlier workers supported the work of present researcher.

A process for the pathway of chromosomal variability into natural plants population can be visualized by Orton (1980) and confirmed that cell initials with lesser degree of chromosomal variability relative to the source culture give rise to chromosomally 'uniform' plants. *In vivo* root tip mitotic study of penda locality of *Centaurea ragusina* seedling exhibiting 9.7% abnormalities were recorded by Radic *et al*. in 2005. The increased stickiness could also lead to the formation of sticky bridges in ana- and telophases, which prevent normal cytokinesis. Radic *et al*. revealed that since chromosome fragments were noticed only in few cells, anaphase and telophases bridges probably resulted from stickiness which is directly supported the present work. However, chromosome stability is an essential prerequisite for maintenance of genetically defined plant material (Jha and Roy 1982). The present worker stated that though abnormalities were recorded at low frequency, their distribution in somatic cells is significant and cannot be overlooked as their transmission into gametes could bring sterility into plants.

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